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COMMUNICATION

Degradation Kinetics of Neostigmine in Solution

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ABSTRACT

The degradation kinetics of neostigmine were studied in aqueous solutions with varied pH from 1.5 to 9.9 under accelerated storage conditions. The stability of neostigmine in solutions containing propylene glycol or polyethylene glycol 400 was also investigated. The reaction order of neostigmine in these aqueous and solvent systems followed pseudo-first-order degradation kinetics. The degradation rates of neostigmine under various buffer concentrations within the investigated pH range were obtained. They indicated that the degradation was independent of the species of buffering agent. Maximum stability of neostigmine was determined at pH 5.0 buffer species conditions. The activation energy could be estimated from the Arrhenius plot as 15.72 kcal/mole. The half-life of 883.7 days was estimated at room temperature in 0.1 M, pH 4.9 acetate buffer solution ($\mu = 0.5$). Ultraviolet (UV) irradiation at 254 nm of the neostigmine solutions in pH 4.9 acetate buffer showed an accelerated degradation in comparison with light-protected samples. Incorporation of propylene glycol into the neostigmine solution at pH 4.9 enhanced the stability; however, an adverse effect on the stability of neostigmine was noted when a polyethylene glycol 400 solvent system was used.

Key Words: *Degradation kinetics; Neostigmine; Stability.*

INTRODUCTION

Neostigmine bromide (3-(dimethylcarbamoyloxy)-*N*,*N*,*N*-trimethylanilinium bromide) is a quaternary amine and acts as an anticholinesterase agent. It acts at the esteratic site of the enzyme to form the inactive dimethylcarbamoyl enzyme. The drug inhibits cholinesterase activity to prolong and intensify the muscarinic and nicotinic effects of acetylcholine. Neostigmine has widespread actions, being particularly effective in the

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bowel, urinary bladder, skeletal muscle, pupil, and heart; also affected are the blood pressure, and secretions are affected in related structures (1). Neostigmine is used mainly for its action on skeletal muscle and less frequently to increase the activity of smooth muscle (2). Neostigmine methylsulfate is used in the treatment of my asthenia gravis, paralytic ileus, postoperative urinary retention, and primary open-angle glaucoma (1).

Neostigmine is absorbed poorly after oral administration, so that much larger doses are needed in comparison to those given by the parenteral route (3). Neostigmine is commercially available as a solution. In aqueous temperature-stressed solutions of neostigmine bromide, besides the known product of hydrolysis, additional degradation products were discovered (4,5). Light irradiation has not been shown to have more effect on the degradation of neostigmine in solution than irradiation with sonic energy. However, it seems that information about its chemical stability is limited. Although determination of neostigmine has been carried out by aqueous titration, potentiometric titration, iodimetric methods, biological methods, colorimetric methods, thin-layer chromatography, spectrophotometry (6), and the high-performance liquid chromatography (HPLC) (3,7), those methods only involved the determination of drug concentration, which may not be suitable for the evaluation of the stability of neostigmine. Thus, the stability-indicating method of neostigmine in solution was important. The objective of this investigation was to develop a novel HPLC method to study the degradation kinetics of neostigmine under various storage conditions, such as pH, total buffer concentration, temperature, light exposure, and cosolvent system.

EXPERIMENTAL

Material

Neostigmine bromide was obtained from Sigma Chemical Company (St. Louis, MO) and was used as received. The HPLC-grade acetonitrile was used in chromatographic determinations. The following analytical-grade chemicals were used as received: propylene glycol, polyethylene glycol 400, ammonium carbonate, acetic acid, sodium acetate, potassium chloride, potassium phosphate monobasic and dibasic, phosphoric acid, sodium hydroxide, boric acid, and sodium borate. Pyridostigmine was used as an internal standard and was obtained from Sigma Chemical Company.

Kinetic Studies

Three hydrochloric acid solutions of various pH from 1.5 to 2.2 and 10 buffer solutions of varied buffer species from pH 3.1 to 9.9 (pH 3.1–4.9 acetate buffer; pH 5.5–8.0 phosphate buffer, and pH 9.1–9.9 borate buffer) were prepared, and the concentration of total buffer species (0.1 M) and ionic strength ($\mu = 0.5$) were fixed. The constant ionic strength was maintained by adjusting the amount of potassium chloride in solution.

Samples of neostigmine at a concentration of 0.4 mg/ml were prepared by dissolving the drug in buffers. All sample solutions were sealed into 2-ml type I flint glass ampoules and stored in a dark oven at 90°C ± 0.2°C for a period of time up to 90 days. At designated storage intervals, samples were removed and immediately stored in a −20°C freezer. At the time of analysis, samples were removed from the freezer, thawed at room temperature, and mixed well before injecting into the HPLC.

A stability-indicating reverse-phase HPLC method was used to determine the concentration of neostigmine. The pH values were measured with a pH meter for each sample to ensure no appreciable pH change. Triplicate samples were used for each storage condition in this study.

High-Performance Liquid Chromatographic Analysis

The HPLC system was composed of a fixed-wavelength UV absorption detector set at 254 nm, a photodiode array UV-Vis detector (model SPD-M6A, Shimadzu Corp., Tokyo, Japan), and a C_{18} column (Nova-Pak, 150 × 3.9 mm id, Millipore Corp., Milford, MA). The mobile phase was a mixture (v/v) of 30% acetonitrile and 70% 0.05 M pentanesulfonate in phosphate buffer (0.1 M) at pH 7.2 to 7.8 adjusted by sodium hydroxide (1.0 N). The mobile phase was delivered at a constant rate of 1.0 ml/min. The absorbance of neostigmine and its internal standard, pyridostigmine (0.04 mg/ml), were recorded by an electronic integrator at a speed of 1.0 cm/min. The concentration of neostigmine was determined to compare the peak area ratio (drug/internal standard) of sample with standard from the calibration curve. In this study, for the determination of the stability at each designated storage time interval, the concentration of neostigmine was expressed as a percentage of its initial concentration (100% at time zero).

Buffer Effect

Three buffer solutions (pH 3.1–4.9 acetate buffer, pH 5.5–8.0 phosphate buffer, and pH 9.1–9.9 borate buffer)

0.05 M, 0.1 M, and 0.2 M with constant ionic strength ($\mu = 0.5$) were prepared (pH 3.1, 0.1 M acetate buffer; pH 6.1, 0.1 M phosphate buffer; and pH 9.1, 0.1 M borate buffer) to study the catalytic effect of buffer species on the degradation of neostigmine (0.4 mg/ml) in aqueous solution at 90°C ± 0.2°C.

Ultraviolet Photolysis Effect

Solutions of 0.1 M pH 4.9 acetate buffer containing 0.4 mg/ml neostigmine were prepared at a constant ionic strength (μ) of 0.5. The solutions were sealed in 2-ml type I flint glass ampoules. Half of the vials were wrapped with aluminum foil to protect the solutions from light and were used as the control group. Both wrapped and unwrapped solutions were under UV light at a distance of 30 cm. The wavelength of the UV light was 254 nm and its intensity was controlled at 150 uW/cm^2. The study was conducted at 25°C ± 0.2°C) for a period up to 90 days.

Temperature Effect

Solutions of 0.1 M pH 4.9 acetate buffer containing 0.4 mg/ml neostigmine were prepared at a constant ionic strength of 0.5. The temperature effect of neostigmine was investigated at 50°C, 60°C, 70°C, 80°C, and 90°C ± 0.2°C.

Solvent Effect

Various solvent systems of propylene glycol:water and polyethylene glycol 400:water with different ratios (10:90, 30:70, 50:50, 70:30) were prepared with a neostigmine concentration of 0.4 mg/ml. The water portion was composed of acetate buffer at pH 4.9. The solvent effect of neostigmine was investigated at a constant temperature of 90°C ± 0.2°C.

RESULTS AND DISCUSSION

High-Performance Liquid Chromatographic Analysis

Some representative chromatograms showing the stability-indicating nature of the developed HPLC method were demonstrated by forcibly degrading 0.4 mg/ml neostigmine solutions (pH 3.1, 0.1 M acetate buffer; pH 6.1, 0.1 M phosphate buffer; and pH 9.1, 0.1 M borate buffer) at 90°C ± 0.2°C for 10 days (Fig. 1). After the accelerated storage, the peak height of neostigmine decreased without apparent interference from the decomposition products. The retention times of neostigmine and pyridostigmine in the HPLC system were 8.57 and 14.49 minutes, respectively. The peak purity of the neostigmine was examined by a photodiode array UV-Vis detector by comparing the UV spectra with that of the pure drug. A lack of difference in the UV spectra between these two samples indicated the absence of interfering decomposition products or exogenous impurities eluting under the peak of interest. The linearity of the calibration curve of

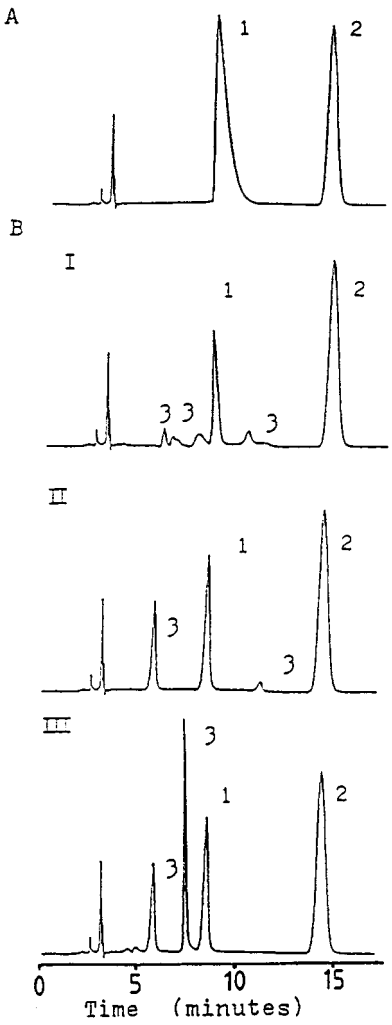

Figure 1. HPLC chromatogram of neostigmine (0.4 mg/ml) in solvent I (pH 3.1, 0.1 M acetate buffer), II (pH 6.1, 0.1 M phosphate buffer), and III (pH 9.1, 0.1 M borate buffer): (A) immediately after preparation and (B) after storage at 90°C ± 0.2°C for 10 days. Key: 1, neostigmine; 2, pyridostigmine; 3, degradation products.

peak area ratio versus neostigmine concentration in the range 0.01 mg/ml to 0.16 mg/ml was demonstrated by an excellent correlation coefficient γ of 0.998 ($Y = 25.95X + 0.04$). The within-day and between-day precision ($n = 5$) of the HPLC method with the concentration of 0.01 mg/ml to 0.1 mg/ml were shown by the coefficient of variation (CV) of 0.97% ± 0.46% and 1.26% ± 0.48%, respectively. The detection limit was 50 ng/ml.

Degradation Kinetics

Stability profiles for neostigmine in 0.1 M buffer solutions at 90°C ± 0.2°C are shown in Fig. 2. The linear relationship between logarithmic percentage remaining and storage time indicated pseudo-first-order degradation kinetics for neostigmine in an aqueous solution; the degradation rate constant was determined from the slope of the graph by the statistical regression analysis method. All regression lines of all studied solutions are linear with correlation coefficients γ greater than 0.98. Porst and Kny (4,5) reported that the concentration course of the degradation products of aqueous temperature-stressed solutions of neostigmine bromide shows that it simultaneously is hydrolyzed to 3-hydroxyphenyltrimethylammonium bromide and demethylated to 3-dimethylaminophenyl-dimethylcarbamate. The ester hydrolysis is the rate-determining predominant reaction and is pseudo-first-order. On the basis of the kinetic results, the possible reaction mechanisms and the influence of selected components on the rate of the hydrolysis and demethylation were also reported.

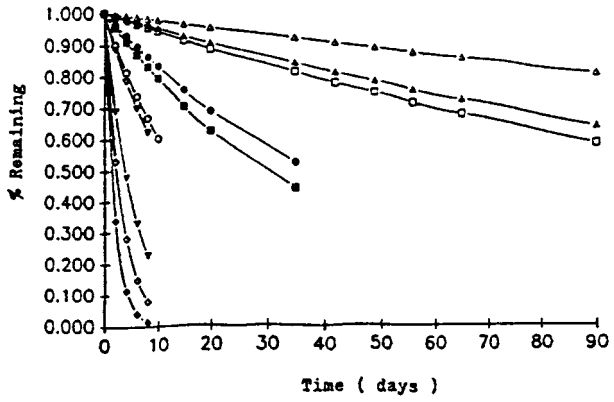

Figure 2. Pseudo-first-order degradation of neostigmine in various buffer solutions (0.1 M) and pH at 90°C ± 0.2°C with ionic strength 0.5: ○, pH 3.1; ●, pH 3.7; △, pH 4.3; ▲, pH 4.9; □, pH 5.5; ■, pH 6.1; ▽, pH 6.7; ▾, pH 8.0; ◇, pH 9.1; ◆, pH 9.9.

Buffer Species

The effect of general acid/base catalysis caused by acetate, phosphate, and borate buffers on the degradation kinetics of neostigmine was studied by varying the total buffer species concentration in solutions while maintaining the pH, ionic strength ($\mu = 0.5$), and storage temperature (90°C ± 0.2°C) constant. No significant difference was observed for the effect of acetate, phosphate, and borate species on the degradation rate constant of neostigmine under three different total buffer concentrations (0.05 M, 0.1 M, and 0.2 M) (Table 1). The degradation of neostigmine in solutions is independent of the species of the buffering agent.

pH Rate Profile

The effect of pH on the degradation of neostigmine in aqueous solution under 0.1 M buffer concentration and constant ionic strength ($\mu = 0.5$) at 90°C ± 0.2°C is shown in Fig. 3, in which the log k_{obs} is plotted against pH. The V-shaped graph was obtained. In the pH range 4.9 to 5.5, neostigmine was found to be more stable than in other pH regions. The degradation of neostigmine was mainly due to the effect of acid catalysis at pH 1.5–3.7. The degradation of neostigmine is described by the catalytic effect of acid and water; however, the base catalysis became more predominant as the pH increased from pH 6.7 to pH 9.9. Equation 1 describes the degradation rate of neostigmine as a function of pH:

$$k_{obs} = k_o + k_H[H^+]^m + k_{OH}[OH^-]^n \quad (1)$$

Table 1

Degradation Rate Constants of Neostigmine in Buffered Solution with Different Buffer Species Concentrations and Constant Ionic Strength ($\mu = 0.5$) and Storage Temperature (90°C ± 0.2°C)

	$k_{obs} \times 10^{-2}$ (1/day)		
pH	0.05 M	0.1 M	0.2 M
3.1	5.20	5.21	5.22
3.7	1.86	1.87	1.88
4.3	1.03	1.04	1.05
4.9	0.52	0.51	0.52
5.5	0.59	0.61	0.62
6.1	1.51	1.51	1.51
6.7	3.52	3.52	3.61
9.1	32.10	31.80	31.80
9.9	53.90	54.20	54.70

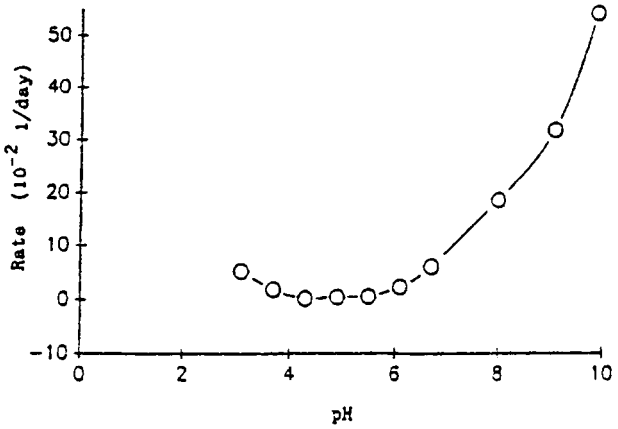

Figure 3. The pH–log (rate) profile of neostigmine in aqueous solution with constant ionic strength ($\mu = 0.5$) and buffer concentration (0.1 M) at 90°C ± 0.2°C. ○, k_{obs}; —, computer simulated.

where k_{obs} = overall observed rate constant, k_o = water catalysis rate constant, k_H = specific acid catalysis rate constant, k_{OH} = specific base catalysis rate constant, and m, n = order of reaction with respect to $[H^+]$ and $[OH^-]$.

Variables in the above rate equation were determined by fitting the rate constants under 0.1 M buffer concentration to the equation. Specific acid and base catalysis rate constants k_H and k_{OH}, respectively, were obtained from the intercepts of log k_{obs} versus pH or pOH plots in the low (pH $<$ 3.1) or high (pH $>$ 8.0) pH regions, respectively. They are $k_H = 1.02 \times 10^{-3}$ M/day and $k_{OH} = 1.7 \times 10^{-3}$ M/day. The values of m and n were determined to be 5.6 and 3.4, respectively, from the slopes of the two pH regions of the V graph. The nonintegral orders of m and n might imply the existence of some intermediate formations during the acid-base catalytic degradation sequences of neostigmine. The rate constant for the water catalysis k_o was determined to be 6.35×10^{-6} L/day from the intersections above two regression lines. The most stable pH based on this calculation was found to be 5.0. Since K_{H+} is greater than K_{OH-}, a faster degradation rate in acidic regions than in alkaline environment is anticipated. A computer simulation of pH-log(rate) profile based on Eq. 1 in comparison with observed rate constants is also shown in Fig. 3.

Temperature Effect

The temperature dependence of neostigmine degradation at pH 4.9, 0.1 M acetate buffer solution ($\mu = 0.5$) was studied at the temperature range 50°C–90°C. The

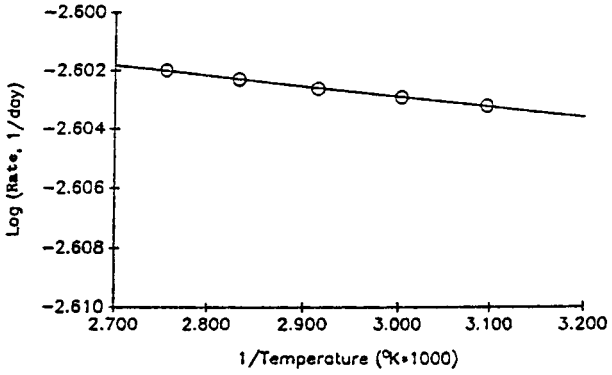

Figure 4. The Arrhenius plot of the degradation of neostigmine (0.4 mg/ml) in the pH 4.9 acetate buffer (0.1 M) solutions and at a constant ionic strength of 0.5.

Arrhenius plot of log(rate constant) versus the reciprocal of absolute temperature (in K) is shown in Fig. 4. The linearity ($\gamma = 0.9978$) of the plot had a good indication of invariant activation energy for degradation of neostigmine in the temperature range 50°C–90°C. The activation energy of degradation was determined to be 15.72 kcal/mol from the slope of this plot. If the activation energy for degradation maintained constant at the temperature range 25°C to 90°C, the degradation rate constant of neostigmine in the acetate buffer at room temperature could be estimated as 7.84 × 10−4 L/day, which gave an estimation of a half-life of 883.9 days.

Ultraviolet Light Effect

After UV irradiation, a significant difference (Student t test, $p < .01$) between light-protected and light-exposed

Table 2

Effect of Ultraviolet Light on the Stability of Neostigmine in pH 4.9 Acetate Buffer Solutions (0.1 M) at $\mu = 0.5$ and 25°C ± 0.2°C

Time (Day)	Remaining Percentage	
	Light Protected	Light Exposed
5	99.7 ± 0.2	98.2 ± 0.2
10	99.5 ± 0.3	97.5 ± 0.3
20	98.7 ± 0.2	95.2 ± 0.2
30	98.3 ± 0.1	94.4 ± 0.3
50	98.1 ± 0.2	91.7 ± 0.2
70	97.8 ± 0.3	87.6 ± 0.4
90	87.6 ± 0.4	85.4 ± 0.3

Table 3

Stability of Neostigmine in Propylene Glycol: Water or Polyethylene Glycol 400: Water Solvent Systems at pH 4.9 and 90°C ± 0.2°C

Solvent Ratio	$k_{obs} \times 10^{-4}$ (L/day)	
	PG:Water	PEG 400:Water
10:90	12.82	87.2
30:70	9.43	173.7
50:50	8.03	348.6
70:30	7.81	392.4

PG = propylene glycol; PEG = polyethylene glycol.

neostigmine solutions was observed. The results are listed in Table 2. No attempt was made to determine the degradation order as the extent of decomposition was too low for reliable estimates. However, UV irradiation did accelerate the degradation processes of neostigmine in the light-exposed samples. Although the actual mechanism for the photolysis of neostigmine due to UV irradiation is not yet clear, the photoionization may be possible.

Solvent Effect

The effects of propylene glycol/polyethylene glycol 400 on the stability of neostigmine in the pH 4.9 acetate buffer solutions at 90°C ± 0.2°C are listed in Table 3. The degradation of neostigmine in these solvent systems followed pseudo-first-order kinetics. It was observed that the degradation rate constant of neostigmine in the propylene glycol:water system decreased as the content of propylene glycol increased. In contrast, the degradation rate constant of neostigmine in the polyethylene glycol 400:water system increased as the polyethylene glycol 400 content increased. No correlation between the degradation rate constant and dielectric constant was observed for the degradation of neostigmine in these solvent systems. The mechanism of the effect of propylene glycol and polyethylene glycol 400 on the degradation of neostigmine is not yet clear.

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